

Serum Vitamin D-binding Protein is a Third Member of the Albumin and Alpha Fetoprotein Gene Family

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Abstract

A near full-length cDNA encoding the human vitamin D-binding protein (hDBP) was isolated from a human liver mRNA expression library. Complete sequence analysis of this clone predicts the full-length amino acid sequence of the pre-hDBP. Comparison of the sequence of the hDBP mRNA and protein to existing protein and nucleic acid data banks demonstrates a strong and highly characteristic homology of the hDBP with human albumin (hALB) and human α -fetoprotein (hAFP). Based upon this structural comparison, we establish that DBP is a member of the ALB and AFP gene family.

Introduction

Human vitamin D-binding protein (hDBP),¹ also known as G-globulin, is an abundant, multifunctional, and highly polymorphic serum glycoprotein synthesized by the liver (1). DBP is the major serum transport protein for the vitamin D sterols (2), binds and sequesters monomers of actin with high affinity (3, 4), and has been identified on the surface of a variety of cell types including B-lymphocytes (5), subpopulations of T-lymphocytes (6), and the cytotrophoblasts of the placenta (7). The physiologic importance of these functions and their possible interrelationships remains to be defined. As an initial step in the detailed study of this abundant serum protein, we have isolated a near full-length copy (cDNA) of the hDBP mRNA, determined the primary structure of the encoded protein, and detected a close evolutionary and genetic relationship between DBP and two other abundant serum proteins, albumin (ALB) and α -fetoprotein (AFP).

Methods

RNA isolation and gel analysis. Total cellular RNA was isolated by standard technique (8) from human liver obtained at autopsy, from fresh rat liver (Sprague-Dawley), and from Hep 3B cells (9) grown in monolayer

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Received for publication 23 September 1985.

1. Abbreviations used in this paper: AFP, α -fetoprotein; ALB, albumin; hDBP, human vitamin D-binding protein.

J. Clin. Invest.

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0021-9738/85/12/2420/05 \$1.00

Volume 76, December 1985, 2420-2424

culture. PolyA+ mRNA was isolated from the total RNA by oligo-dT chromatography (10). The mRNAs were size-fractionated by electrophoresis through a 1% agarose-formaldehyde gel (11), transferred to nitrocellulose paper (12), and hybridized at reduced stringency (37°C with other conditions, as described in reference 12) to a nick-translated ³²P-labeled cDNA probe (13). After overnight hybridization, the filter was washed (at 42°C, otherwise as described in reference 12) and autoradiographed at -70°C on XAR film (Eastman Kodak Co., Rochester, NY) in the presence of a Cronex Lightening Plus intensifying screen. (E. I. DuPont de Nemours, Wilmington, DE). Ribosomal RNA from human reticulocytes (28S and 18S) and *Escherichia coli* (23S and 16S) were run on the gel in separate lanes, visualized by ethidium bromide staining, and used as molecular weight markers.

cDNA library screening. A library of human liver cDNA inserted in the expression vector λ gt11 (14) was a generous gift of S. Woo, Howard Hughes Medical Institute, Baylor College of Medicine, Texas Medical Center, Houston, TX (15). This library was plated out on Y1090 bacteria and the plaques were screened by the method of Benton and Davis (16). All positives were plaque-purified by serial platings at low dilution. cDNA inserts were restriction mapped from phage DNA isolated by a mini-lysates procedure (17).

cDNA subcloning. DNA from each phage mini-lysate was digested with the restriction endonuclease EcoRI (New England Biolabs, Beverly, MA) at 1 U/ μ g DNA under conditions suggested by the manufacturer.

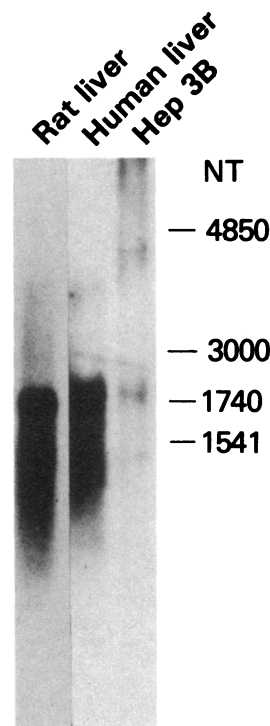


Figure 1. Detection of the human DBP mRNA by cross hybridization with a rat DBP cDNA probe. 5 μ g of polyA+ mRNA from rat liver, 5 μ g of polyA+ mRNA from human liver, or 20 μ g of total RNA from Hep 3B cells were electrophoresed through a 1% agarose-formaldehyde gel, transferred to nitrocellulose paper, and hybridized at reduced stringency to a nick-translated, ³²P-labeled rDBP cDNA probe (Cooke, N. E., unpublished data). An autoradiograph of the hybridized filter is shown with the lanes identified. The position of ribosomal RNA molecular size markers are indicated in nucleotides (NT).

C	GGT	GCT	GCA	AGA	CTC	TCT	GGT	AGA	AAA	-16 Met	Lys	Arg	Val	Leu	Val	-10 Leu	Leu	Leu	Ala	Val	Ala	Phe	Gly	His	-1 Ala	+1 Leu	Glu	Arg	Gly	98
Arg	Asp	Tyr	Glu	Lys	Asn	Lys	Val	Cys	Lys	Glu	Phe	Ser	His	Leu	20 Gly	Lys	Glu	Asp	Phe	Thr	Ser	Leu	Ser	Leu	30 Val	Leu	Tyr	Ser	Arg	178
Lys	Phe	Pro	Ser	Gly	Thr	Phe	Glu	Gln	Val	Ser	Gln	Leu	Val	Lys	50 Glu	Val	Val	Ser	Leu	Thr	Glu	Ala	Cys	Cys	Ala	60 Glu	Gly	Ala	Asp	268
Pro	Asp	Cys	Tyr	Asp	Thr	Arg	Thr	Ser	Ala	Leu	Ser	Ala	Lys	Ser	80 Glu	Glu	Ser	Asn	Ser	Pro	Phe	Pro	Val	His	90 Pro	Gly	Thr	Ala	Glu	358
Cys	Cys	Thr	Lys	Glu	Gly	Leu	Glu	Arg	Lys	Leu	Cys	Met	Ala	Ala	110 Leu	Lys	His	Gln	Pro	Gln	Glu	Phe	Pro	Thr	120 Tyr	Val	Glu	Pro	Thr	448
Asn	Asp	Glu	Ile	Cys	Glu	Ala	Phe	Arg	Lys	Asp	Pro	Lys	Glu	Tyr	140 Ala	Asn	Gln	Phe	Met	Trp	Glu	Tyr	Ser	Thr	150 Asn	Tyr	Glu	Gln	Ala	538
Pro	Leu	Ser	Leu	Leu	Val	Ser	Tyr	Thr	Lys	Ser	Tyr	Leu	Ser	Met	170 Val	Gly	Ser	Cys	Cys	Thr	Ser	Ala	Ser	Pro	180 Thr	Val	Cys	Phe	Leu	628
Lys	Glu	Arg	Leu	Gln	Leu	Lys	His	Leu	Ser	Leu	Thr	Thr	Leu	200 Ser	Asn	Arg	Val	Cys	Ser	Gln	Tyr	Ala	Ala	210 Tyr	Gly	Glu	Lys	Lys	718	
Ser	Arg	Leu	Ser	Asn	Leu	Ile	Lys	Leu	Ala	Gln	Lys	Val	Pro	Thr	230 Ala	Asp	Leu	Glu	Asp	Val	Leu	Pro	Leu	Ala	240 Glu	Asp	Ile	Thr	Asn	888
Ile	Leu	Ser	Lys	Cys	Glu	Ser	Ala	Ser	Glu	Asp	Cys	Met	Ala	260 Lys	Glu	Leu	Pro	Glu	His	Thr	Val	Lys	Leu	270 Cys	Asp	Asn	Leu	Ser	898	
Thr	Lys	Asn	Ser	Lys	Glu	Glu	Asp	Cys	Cys	Gln	Glu	Lys	Thr	Ala	290 Met	Asp	Val	Phe	Val	Cys	Thr	Tyr	Phe	Met	300 Pro	Ala	Ala	Gln	Leu	988
Pro	Glu	Leu	Pro	Asp	Val	Arg	Leu	Pro	Thr	Asn	Lys	Asp	Val	Cys	320 Asp	Pro	Gly	Asn	Thr	Lys	Val	Met	Asp	Lys	330 Tyr	Thr	Phe	Glu	Leu	1078
Ser	Arg	Arg	Thr	His	Leu	Pro	Glu	Val	Phe	Leu	Ser	Lys	Val	Leu	350 Glu	Pro	Thr	Leu	Lys	Ser	Leu	Gly	Glu	Cys	360 Tyr	Asp	Val	Glu	Asp	1168
Ser	Thr	Thr	Cys	Phe	Asn	Ala	Lys	Gly	Pro	Leu	Leu	Lys	Lys	Glu	380 Leu	Ser	Ser	Phe	Ile	Asp	Lys	Gly	Gln	Glu	390 Cys	Cys	Ala	Asp	Tyr	1258
Ser	Glu	Asn	Thr	Phe	Thr	Glu	Tyr	Lys	Lys	Lys	Leu	Ala	Glu	Arg	410 Leu	Lys	Ala	Lys	Leu	Pro	Glu	Ala	Thr	Pro	420 Glu	Glu	Leu	Ala	Lys	1348
Leu	Val	Asn	Lys	Arg	Ser	Asp	Phe	Ala	Ser	Asn	Cys	Cys	Ser	Ile	440 Asn	Ser	Pro	Pro	Leu	Tyr	Cys	Asp	Ser	Glu	450 Ile	Asp	Ala	Glu	Leu	1438
Lys	Asn	Ile	Leu	—	—	—	—	—	—	—	—	—	—	—	458 Asn	Ser	Pro	Pro	Leu	Tyr	Cys	Asp	Ser	Glu	458 Ile	Asp	Ala	Glu	Leu	1528
CAA	AAC	CAC	TGA	GCT	TCT	GGG	AAG	ACA	ACT	AGG	ATA	CTT	TCT	ACT	TTT	TCT	AGC	TAC	AAT	ATC	TTC	ATA	CAA	TGA	CAA	GTA	TGA	TGA	TTT	1618
GCT	ATC	AAA	ATA	AAT	TGA	AAT	ATA	ATG	CAA	ACC	ATA	Poly A																		1654

Figure 2. The nucleotide sequence of the hDBP cDNA and the full-length predicted amino acid sequence of hDBP. Nucleotides are numbered beginning with the first base of the hDBP cDNA insert. Amino acids are numbered with 1 representing the first residue of the mature protein.

The digest was phenol-extracted and ligated to EcoRI-digested and dephosphorylated SP65 plasmid (18) at a 5:1 molar ratio of insert to vector using 400 U of T4 ligase (New England Biolabs) in a total volume of 20 μ l containing 50 mM Tris HCl, pH 7.4, 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM spermidine, and 1 mM ATP. The entire ligation reaction was used to transform *E. coli* HB101 rendered competent by cold CaCl₂ treatment (19). Transformations were spread on L-broth plates containing ampicillin (35 μ g/ μ l). Ampicillin-resistant transformants were screened in situ (20) with ³²P-labeled cDNA.

Plasmid preparation. All recombinant work was done under P1 containment conditions. Bacterial plasmids were grown in 1-liter batches to an OD₆₀₀ of 0.8, amplified with chloramphenicol (170 μ g/ μ l), and harvested 18 h later. Supercoiled plasmid was purified from a clarified lysate by cesium chloride isopycnic centrifugation in the presence of 0.75 mg/ml ethidium bromide, harvested, and phenol-extracted before use.

DNA sequencing. Restriction sites with 5' protruding ends were dephosphorylated with calf intestinal alkaline phosphatase (Bethesda Research Laboratories, Gaithersburg, MD) and ³²P end-labeled in a 25- μ l reaction with T4 polynucleotide kinase (New England Biolabs) and (³²P)rATP (5,000 Ci/mM, Amersham Corp., Arlington Heights, IL). 3' recessed ends were labeled with *E. coli* DNA polymerase, Klenow fragment (Bethesda Research Laboratories), and the appropriate (³²P)dNTP in the recommended buffer. End-labeled fragments were either strand-separated (21) or digested with a second restriction enzyme before gel purification and sequencing. All DNA sequencing was by the method of Maxam and Gilbert (22) with modifications as previously described (23). All regions were either sequenced on both strands, or two separate times on the same strand.

Primer extension analysis. For primer extension analysis a gel-purified end-labeled ³²P cDNA fragment was hybridized to liver mRNA in 30 μ l

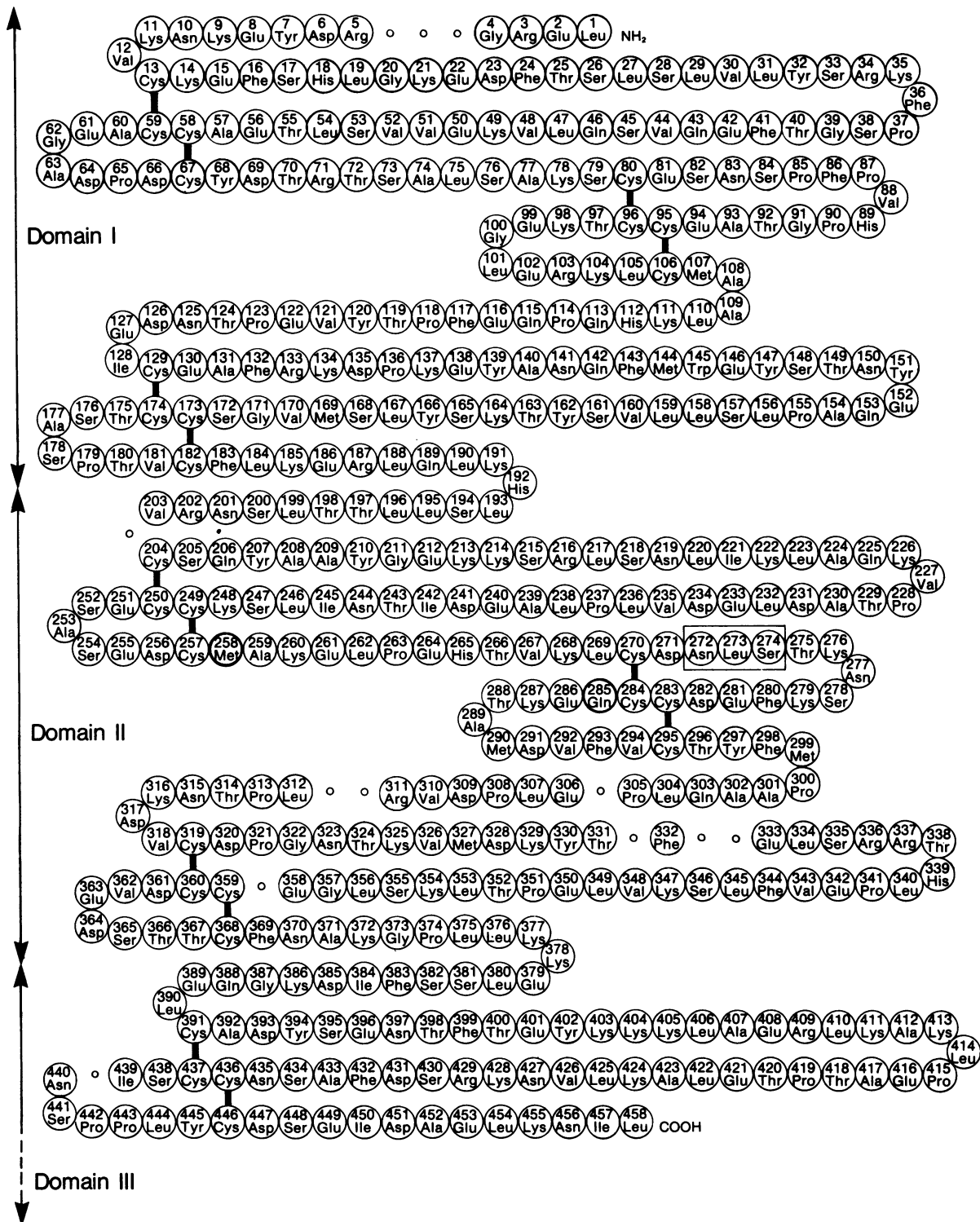


Figure 3. Conservation of cysteine positioning in hDBP and hALB. The predicted amino acid sequence of hDBP is displayed in the format originally proposed by J. R. Brown for albumin (30). Deletions in hDBP, when aligned with hALB, are indicated by the small circles,

and insertions by bold outline. A potential N-linked glycosylation site is enclosed in a rectangle. The position of the homologous internal domains is indicated in the left margin.

of hybridization buffer (80% deionized formamide, 0.4 M NaCl, 40 mM PIPES, pH 6.4, 1 mM EDTA) at 42°C for 3 h. Primer extension with avian myeloblastosis virus reverse transcriptase was subsequently carried out as previously described (24) with no significant modification.

Computer analysis. GenBank, European Molecular Biology Labs, and National Biomedical Research Foundation databases and software used in the data analysis were accessed through the Bionet National Computer Resource for Molecular Biology, Palo Alto, CA.

Results

A rat DBP cDNA clone (Cooke, N. E., unpublished data), selected by an immunological approach (to be described elsewhere), was shown by Northern analysis to cross-hybridize under conditions of reduced stringency to a single mRNA species of 1,750 nucleotides from the human liver (Fig. 1). This same mRNA is present in the human hepatoma cell line Hep 3B which is known to synthesize DBP (25) but not in human placental mRNA (data not shown). A slightly smaller mRNA species of 1,650 nucleotides is seen in the rat liver mRNA. Utilizing this low stringency cross-hybridization between the rDBP cDNA and the hDBP mRNA, several human liver DBP cDNA clones were selected from a human liver cDNA library constructed in the bacteriophage λ gt11 (15). The cDNA insert in each of the hybridization-positive phage clones was mapped, and the largest, 1,383 nucleotides long, was fully restriction-mapped and sequenced. The identity of this clone as encoding hDBP was established by identification of three previously sequenced peptide fragments of hDBP (amino acids 1–41 [26], 414–419, and 421–424 [27]) and by 77% homology to the terminal 441 amino acids of the rDBP cDNA clone. Using the 5' EcoRI fragment of this partial clone as probe, an overlapping hDBP cDNA clone extending further in the 5' direction was selected and restriction-mapped. This recombinant contained a 1,654 base-pair cDNA insert. The sequence of this human cDNA, shown in Fig. 2, contains a 1,422 nucleotide open reading frame beginning with a Met codon at the 29th base.

To establish whether the cDNA displayed in Fig. 2 was full-length, a 107 base-pair restriction fragment labeled at the EcoRI site (nucleotide 119) and extending to the Hinfl site (nucleotide 12) was isolated from the 5' end of the cDNA, and hybridized to human liver RNA. cDNA synthesis was extended with reverse transcriptase. The resultant cDNA, sized next to a DNA sequence ladder, extended 48 nucleotides 5' to the labeled fragment (data not shown). Thus the reported cDNA sequence begins 36 base pairs 3' to the mRNA cap site and the full-length hDBP mRNA is predicted to contain 1,690 nucleotides, excluding its polyA⁺ tail.

Discussion

The full-length primary sequence of hDBP, as displayed in Fig. 2, can be correlated with several of the known physical properties

of this protein. DBP is a secreted protein and would therefore be predicted to have a signal sequence. The amino-terminus of the mature serum protein was established by aligning the sequence predicted by the cDNA to a sequenced amino-terminal hDBP peptide (27). From this alignment we deduce that the coding region of prehDBP begins with a 16-amino-acid hydrophobic signal sequence. Human DBP is known to be glycosylated and the determined sequence contains one potential N-linked glycosylation site (Fig. 3, enclosed in rectangle). The encoded mature hDBP contains 458 residues with a calculated molecular weight of 51,335, compared with experimental estimates of 54,000–58,000 for the serum glycoprotein.

DBP, ALB, and the fetal analogue of ALB, AFP, are major serum proteins synthesized by mammalian liver parenchymal cells. All three molecules possess a high content of cysteine residues. Comparison of the cysteines in hDBP to those in hALB and hAFP reveals a high degree of conservation in their number and positions. An alignment of hDBP, hALB (28), and hAFP (29) using the cysteine residues as landmarks, revealed significant additional amino acid and nucleotide homologies (Table I). The similarities between these three molecules are so strong that hDBP can be easily displayed in the format proposed by J. R. Brown (30) for albumin (Fig. 3). Human DBP terminates 123 amino acids before hALB or hAFP, and there is a rapid drift in the 3' untranslated region of hDBP compared with the continued coding regions of the other two mRNAs. Human DBP contains a unique tryptophan at position 145 not present in hALB, or hAFP. The glycosylation pattern of the three proteins appears to differ: the predicted glycosylation sites in hAFP are not conserved in hDBP, and hALB is not glycosylated at all.

The triplicated internal domain structure first noted in ALB and AFP is also present in hDBP (Table I) with the exception of the previously mentioned truncation within the third domain of hDBP. This suggests a common evolutionary origin of these three serum proteins from a precursor with a triplication of an original single domain. The hDBP gene may in fact be situated in close proximity to the hALB and hAFP genes. Previous studies of families with albumin mutations have documented a linkage of <1.5 centimorgan map units between the hDBP and the hALB loci on human chromosome 4 in segment 4q11–13 (31–34). This localization and linkage has been recently confirmed by *in situ* hybridization using the hDBP cDNA as probe (Cooke, N. E., H. Willard, E. V. David, and D. George, unpublished

Table I. Amino Acid and Nucleotide Sequence Comparisons between hDBP, hALB, hAFP, and Internal Domains

Comparison	Gaps	Amino acids				Total* percent	Gaps	Coding nucleotides	
		Identical		Conservative replacement (35)				Homology	
		No.	Percent	No.	Percent			No.	Percent
DBP/ALB (28)	14	112/487	23.0	124/487	25.4	48.5	42	570/1,461	39.0
DBP/AFP (29)	16	95/448	19.5	118/488	24.2	43.7	48	536/1,464	36.6
ALB/AFP	12	244/616	39.6	160/616	26.0	65.6	36	953/1,848	51.6
DBP internal domains									
I/II	10	45/194	23.2	43/194	22.2	45.4	30	224/582	38.5
I/III	1	12/81	14.8	20/81	24.6	39.4	3	79/243	32.5
II/III	3	15/81	18.5	22/81	27.2	45.7	9	89/243	36.6

Gaps were inserted to maximize amino acid homology. The signal peptides are included in the amino acid analyses. Total lengths include gaps which are treated as mismatches. * Total includes identical and conservative replacements.

data). Based upon the primary structural homologies at both mRNA and protein levels, the presumed similarities in the secondary structures indicated by strictly conserved placement of cysteine residues, and the linkage of the three encoding genes on chromosome 4, we conclude that DBP is a member of the gene family that encodes the other major serum proteins, ALB and AFP.

Note added in proof. While this manuscript was in press, we learned of the work of Yang et al. (36) in which a DBP cDNA of the Gc² type was characterized. Our sequence differs from theirs in positions 152, 311, 416, and 420. The latter two amino acids of our sequence are consistent with those reported in Gc by Svasti et al. (27) and suggest that our sequence represents the Gc¹ allele.

Acknowledgments

This work was supported by National Institutes of Health research grant RO1GM32035 (Dr. Cooke). Dr. Cooke is a recipient of a Basil O'Connor Starter Grant from the National Foundation—March of Dimes.

References

- Haddad, J. G. 1979. Transport of vitamin D metabolites. *Clin. Orthop. Relat. Res.* 142:249–274.
- Daiger, S. P., M. S. Schanfield, and L. L. Cavalli-Sforza. 1975. Group specific component (Gc) proteins bind vitamin D and 25-hydroxyvitamin D. *Proc. Natl. Acad. Sci. USA.* 72:2076–2080.
- Cooke, N. E., J. Walgate, and J. G. Haddad. 1979. Human serum binding protein for vitamin D and its metabolites. I. Physicochemical and immunological identification in human tissues. *J. Biol. Chem.* 254:5965–5971.
- Van Baelen, H., R. Bouillon, and P. DeMoor. 1980. Vitamin D binding protein (Gc-Globulin) binds actin. *J. Biol. Chem.* 255:2270–2272.
- Petrini, M., D. L. Emerson, and R. M. Galbraith. 1983. Linkage between surface immunoglobulin and cytoskeleton of B lymphocytes may involve Gc protein. *Nature (Lond.)* 306:73–74.
- Petrini, M., R. M. Galbraith, D. L. Emerson, A. E. Nel, and P. Arnaud. 1985. Structural studies of T lymphocyte Fc receptors. *J. Biol. Chem.* 260:1804–1810.
- Nestler, J. E., E. Lange, J. McLeod, H. J. Kliman, and J. F. Strauss. 1985. Detection of vitamin D binding protein (DBP) in human cytotrophoblasts. *Seventh Annual American Society for Bone and Mineral Research.* Wash. DC. 266a. (Abstr.).
- Chirgwin, J., A. E. Przybyla, R. S. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294–5301.
- Knowles, B. B., C. Howe, and D. P. Aden. 1980. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science (Wash. DC)* 209:497–499.
- Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. USA.* 69:1408–1412.
- Rave, N., R. Crkvenjakov, and H. Boedtker. 1979. Identification of procollagen mRNAs transferred to diazobenzoyloxymethyl paper from formaldehyde agarose gels. *Nucl. Acids Res.* 6:3559–3567.
- Thomas, P. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* 77:5201–5205.
- Maniatis, T., A. Jeffrey, and D. G. Kleid. 1975. Nucleotide sequence of the rightward operator of Phage λ . *Proc. Natl. Acad. Sci. USA.* 72:1184–1188.
- Young, R. A., and R. W. Davis. 1983. Yeast RNA polymerase II genes: isolation with antibody probes. *Science (Wash. DC)* 222:778–782.
- Kwok, S. C. M., F. D. Ledley, A. G. DiLella, K. J. H. Robson, and S. L. C. Woo. 1985. Nucleotide sequence of a full-length complementary DNA clone and amino acid sequence of human phenylalanine hydroxylase. *Biochemistry.* 24:556–561.
- Benton, W. D., and R. W. Davis. 1977. Screening λ gt recombinant clones by hybridization to single plaques in situ. *Science (Wash. DC)* 196:180–182.
- Leder, P., D. Tiemeier, and L. Enquist. 1977. EK2 derivatives of bacteriophage lambda useful in the cloning of DNA from higher organisms: the λ gtWES system. *Science (Wash. DC)* 196:175–177.
- Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucl. Acids Res.* 12:7035–7054.
- Dagert, M., and S. D. Ehrlich. 1979. Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. *Gene (Amst.)* 6:23–28.
- Grunstein, M., and D. Hogness. 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. *Proc. Natl. Acad. Sci. USA.* 72:3961–3965.
- Maxam, A. M., and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific cleavage reactions. *Methods Enzymol.* 65:499–560.
- Maxam, A. M., and W. Gilbert. 1977. A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA.* 74:560–564.
- Cooke, N. E., D. Coit, R. I. Weiner, J. D. Baxter, and J. A. Martial. 1980. Structure of the cloned DNA complementary to rat prolactin messenger RNA. *J. Biol. Chem.* 255:6502–6510.
- Liebhaber, S. A., and Y. W. Kan. 1981. Differentiation of the mRNA transcripts originating from the $\alpha 1$ and $\alpha 2$ globin loci in normals and α -thalassemics. *J. Clin. Invest.* 68:439–446.
- Haddad, J. G., D. P. Aden, and M. A. Kowalski. 1983. Characterization of the human plasma binding protein for vitamin D and its metabolites synthesized by the hepatoma-derived cell line, Hep 3B. *J. Biol. Chem.* 258:6850–6854.
- Schoentgen, F., M. H. Metz-Boutigue, J. Jolles, J. Constans, and P. Jolles. 1985. Homology between the human vitamin D-binding protein (group specific component), α -fetoprotein and serum albumin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 185:47–50.
- Svasti, J., A. Kurosky, A. Bennett, and B. H. Bowman. 1979. Molecular basis for the three major forms of human serum vitamin D binding protein (group specific component). *Biochemistry.* 18:1611–1617.
- Dugaiczky, A., S. W. Law, and O. E. Dennison. 1982. Nucleotide sequence and the encoded amino acids of human serum albumin mRNA. *Proc. Natl. Acad. Sci. USA.* 79:71–75.
- Morinaga, T., S. Masaharu, T. G. Wegman, and T. Tamaoki. 1983. Primary structures of human α -fetoprotein and its mRNA. *Proc. Natl. Acad. Sci. USA.* 80:4604–4608.
- Brown, J. R. 1976. Structural origins of mammalian albumin. *Fed. Proc.* 35:2141–2144.
- Weitkamp, L. R., D. L. Rucknagel, and H. Gershowitz. 1966. Genetic linkage between structural loci for albumin and group specific component (Gc). *Am. J. Hum. Genet.* 18:559–566.
- Kaarsalo, E., L. Melartin, and B. S. Blumberg. 1967. Autosomal linkage between the albumin and Gc loci in humans. *Science (Wash. DC)* 158:123–125.
- Mikkelsen, M., P. Jacobsen, and K. Henningsen. 1977. Possible localization of Gc-system on chromosome 4. *Hum. Hered.* 27:105–107.
- Harper, M. E., and A. Dugaiczky. 1983. Linkage of the evolutionarily-related serum albumin and α -fetoprotein genes within q11–22 of human chromosome 4. *Am. J. Hum. Genet.* 35:565–572.
- Dayhoff, M. O. 1978. Atlas of Protein Sequence. Vol. 5, Suppl. 3. National Biomedical Research Foundation, Washington, DC. 345–352.
- Yang, F., J. L. Brune, S. L. Naylor, R. L. Cupples, K. H. Naberhaus, and B. H. Bowman. 1985. Human group-specific component (Gc) demonstrates striking homology with the albumin family of genes. *Proc. Natl. Acad. Sci. USA.* In press.